Influence of caloric contribution and saturation of dietary fat on plasma lipids in premenopausal women[1–3]

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ABSTRACT Total cholesterol, HDL cholesterol, and triglycerides were measured in 31 premenopausal women randomized into one of two diet groups: one diet with a P:S ratio of 1.0 and one diet with a P:S ratio of 0.3. Both groups were fed a high-fat diet (40% of energy from fat) for four menstrual cycles per subject followed by a similar interval on a low-fat diet (20% of energy from fat). Changing from the high-fat to the low-fat diet resulted in a nonsignificant mean decrease of 7% in total cholesterol. HDL-cholesterol response to the low-fat regimen was influenced by the P:S ratio. Women in the high P:S group showed no change; mean HDL cholesterol in women in the low P:S group decreased 12%. Plasma triglycerides increased in both groups on the low-fat diet although the increase was greatest in the low P:S group. *Am J Clin Nutr* 1987;45:1451–6.

KEY WORDS Cholesterol, triglycerides, polyunsaturated fat, P:S ratio

Introduction

Changes in blood lipid patterns are often taken to reflect alterations in the quantitative and qualitative nature of dietary fat. Formulae have been published predicting plasma cholesterol levels based on dietary cholesterol intake (1, 2). However, most of the research on changes in dietary fat and the resultant effects on blood lipids has involved adult men at risk for heart disease due to elevated serum cholesterol. These studies led to the general recommendation that increasing the ratio of polyunsaturated to saturated fatty acids (P:S ratio) of dietary fat along with concomitant decreases in intake of total fat and cholesterol would decrease plasma cholesterol and thereby reduce the risk of heart disease (3).

The response of plasma lipids to dietary fat intake in groups other than high-risk males remains unclear. Studies with women have been particularly scarce. Some investigators have examined plasma lipids in women fed high- and moderate-fat diets of differing P:S composition (4, 5) or very low fat defined-formula diets (6). Brussaard et al (7, 8) conducted a series of controlled-diet studies with high-, moderate-, and low-fat diets of conventional foods but only the high-fat group also allowed comparison of P:S effects. This report examines plasma lipids in a group of healthy premenopausal women fed high- and low-fat diets of conventional foods, both diets at two levels of saturation of the dietary fat (P:S = 1.0 and P:S = 0.3).

Methods

Premenopausal women aged 20–40 yr were recruited from the Beltsville, MD area to study the effects on various biologic indices of eating high-fat (40% energy from fat) vs low-fat (20% energy from fat) diets at low (0.3) or high (1.0) P:S ratios. This study was reviewed and approved by the human studies review committees of the NIH, USDA, and Georgetown University of Medicine.

Ninety-seven volunteers responded to local advertisements and were prescreened with dietary and medical questionnaires to eliminate women with health problems including metabolic diseases, past or present breast disease, history of thyroid disease, use of oral contraceptives in the past year, regular use of any prescribed or over-the-counter medications, self-reported menstrual irregularities or other reproductive problems, pregnancy or breast-feeding within the last year, and dietary patterns incompatible with the study (eg, vegetarian diets). Forty women meeting the

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stated criteria were further screened with a more detailed medical history, a physical examination, and laboratory testing including a blood cell count, a biochemical profile, and urinalysis. Subjects whose weights were < 90% or > 120% of the 1983 Metropolitan Life Insurance *desirable weights* (9) were excluded. Of the 37 women who passed the screening evaluations and started the study, 31 completed the study. Reasons cited for dropping out were of a personal nature (transportation, $n = 2$; job changes, $n = 3$; and pregnancy, $n = 1$). The data presented here represent only the 31 women who completed the study.

After pairing based on relative weight (wt/ht), the subjects were randomized to one of two dietary groups (P:S ratios = 0.3 or 1.0), which were maintained throughout both the high- and low-fat dietary regimens. This randomization process was subject to the constraint that smokers ($n = 7$) be evenly represented in both P:S groups.

Diets contributing either 40% or 20% energy from fat with P:S ratios of 0.3 and 1.0 were formulated from commonly available foods. Nutrient composition of the diets was calculated using the USDA Lipid Nutrition Laboratory food database, derived from data on food composition from the USDA together with data provided by the food industry, the Nutrient Coding Center in Minneapolis, and by analysis. A 14-d menu cycle was utilized to provide variety and maintain acceptability of the diets. Menus for four caloric intake levels (1600, 2000, 2400, and 2800 kcal) were formulated. Women began the study at the caloric level closest to their estimated maintenance requirement as calculated using the recommended energy intake (10) for women of this age group and adjusting for size and self-reported usual activity level. Subjects were weighed every weekday morning before breakfast. In order to maintain body weight, whenever a subject gained or lost ~1 kg, she was moved from one caloric level to the other. All nutrients for which food data are available were provided by the diet in amounts to meet the Recommended Dietary Allowances (10) for women in the age group of the subjects. In reducing the energy intake from fat, the energy from protein was maintained at ~16–17% while that from carbohydrate was increased. However, the ratio of type of carbohydrate, ie, complex to simple sugars, was maintained at ~1:1. No vitamin or mineral supplements were consumed by the subjects while on the study. The mean daily dietary intake for the two P:S groups during the high- and low-fat periods is shown in **Table 1.**

During the controlled dietary periods, all meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC). On weekdays the morning and evening meals were eaten in the BHNRC dining facility. A carryout lunch was provided. Weekend meals were prepackaged in the Human Study Facility for home consumption and were the same menus as other days of the menu cycle. No foods other than those provided by the study were permitted. Alcohol consumption was not allowed during the controlled dietary periods. An unlimited amount of tea and coffee were allowed provided that sweeteners or other additives were those provided with the study meals and that the amount consumed was recorded.

After a free-living baseline period lasting one menstrual cycle, each woman was placed on the high-fat diet for four menstrual cycles and then switched to the low-fat diet for a similar period of four menstrual cycles. Morning fasting blood samples were collected during two successive 5-d periods (midfollicular and midluteal) of the menstrual cycle during both baseline and last menstrual cycles of the high- and low-fat periods. The first blood sample drawn during the follicular period of the menstrual cycle for each subject was analyzed for plasma total cholesterol, HDL cholesterol, and triglycerides. For the baseline only, total cholesterol in the luteal phase was also analyzed using the last blood sample collected. Plasma cholesterol and triglycerides were measured using enzymatic colorimetric procedures (11, 12). HDL cholesterol was measured using the same cholesterol procedure in the supernatant after a phosphotungstate-magnesium precipitation of the LDL, VLDL, and chylomicrons (13). Analyses were done using frozen stored samples for the baseline period and fresh samples during the high- and low-fat periods.

High- and low-fat intake periods were compared between individuals using paired "Student's" t tests and unpaired "Student's" t tests were used for contrasts across P:S groups. All p-values calculated assumed a two-tailed test and an alpha value of 0.05 was considered statistically significant. Statistical analyses were done using the Statistical Analysis System, SAS (14).

Results

Basic descriptive data, baseline plasma lipids, and baseline dietary intake for the women

TABLE 1
Mean ± SEM daily nutrient intake for subjects in both P:S groups during high- and low-fat dietary periods

	Percent of energy from fat			
	40%		20%	
	P:S ratio		P:S ratio	
	0.3	1.0	0.3	1.0
Energy (kcal)	2278 ± 65	2180 ± 81	2260 ± 97	2208 ± 110
Protein (% kcal)	16%	16%	17%	17%
Carbohydrate (% kcal)	45%	45%	64%	64%
Fat (% kcal)	39%	39%	19%	19%
Cholesterol (mg)	374 ± 11	289 ± 10	230 ± 9	199 ± 9
Saturated fat (g)	44.2 ± 1.3	26.8 ± 1.1	20.9 ± 0.7	12.3 ± 0.6
Oleic acid (g)	30.5 ± 0.9	33.5 ± 1.4	14.9 ± 0.6	17.0 ± 0.8
Linoleic acid (g)	14.6 ± 0.4	26.1 ± 0.9	6.9 ± 0.2	12.9 ± 0.6

TABLE 2
Mean ± SEM physical characteristics, baseline plasma lipids, and baseline dietary intake of study subjects*

	Group (P:S ratio)†		
	0.3	1.0	Total
n	15	16	31
Age (yr)	28.4 ± 1.5	27.1 ± 1.1	27.7 ± 0.9
Height (cm)	159.8 ± 1.5	158.5 ± 2.5	159.2 ± 1.5
Weight (kg)	58.4 ± 2.4	56.0 ± 1.8	57.7 ± 1.5
Cholesterol (mg/dL)	180.4 ± 7.9	195.6 ± 7.1	188.2 ± 5.4‡
HDL cholesterol (mg/dL)	60.3 ± 3.3	62.7 ± 3.9	61.5 ± 2.5
Triglycerides (mg/dL)	49.9 ± 7.7	49.0 ± 5.7	49.4 ± 4.7
Energy (kcal)	2111 ± 65	2012 ± 63	2061 ± 45
Protein (% kcal)	14	14	14
Carbohydrate (% kcal)	45	48	46
Fat (% kcal)	41	38	40
P:S ratio	0.5	0.5	0.5
Cholesterol (mg)	468 ± 31	392 ± 24	429 ± 20

* Baseline nutrient intake was calculated from 7-d food records collected during the middle of the baseline menstrual cycle.

† Data is presented by P:S group to demonstrate the quality of randomization. Subjects were free-living during this baseline period and not consuming the study diets.

‡ In contrast to this follicular measurement, mean cholesterol measured in the luteal phase of the baseline menstrual cycle was 176.3 ± 6.7 mg/dL.

randomized to each P:S group are shown in **Table 2.** No significant differences were seen between P:S groups in any of the indices at baseline, indicating that the randomization was successful. Mean plasma cholesterol was ~12 mg/dL (~0.31 mmol/L) lower in the luteal phase of the baseline menstrual cycle than in the follicular phase.

Plasma lipid measurements obtained during the high- and low-fat dietary regimens are shown in **Table 3.** Although plasma cholesterol values decreased by 7% on the low-fat diet, the change was not statistically significant (mean difference = 13.2 mg/dL (0.34 mmol/L), 95% confidence interval [CI] = −3.9 to 30.3, $p = 0.142$). HDL cholesterol decreased 6% overall though the drop was limited to the low P:S group where the mean value declined by 12% (mean difference = 6.4 mg/dL (0.17 mmol/L), 95% CI = −1.0 to 13.8, $p = 0.118$). Plasma triglycerides were the only lipids among those measured that showed a statistically significant difference between dietary periods. Overall, triglycerides increased by 32% in the low- compared to the high-fat period (mean difference = 13.2 mg/dL (0.15 mmol/

TABLE 3
Mean ± SEM plasma lipid values for high- and low-fat dietary periods by P:S groups

	P:S	*n*	High fat	Low fat	Difference
Cholesterol (mg/dL)	1.0	16	182.1 ± 8.5	167.4 ± 12.1	−14.6 ± 13.6
	0.3	15	188.0 ± 12.0	176.3 ± 12.4	−11.6 ± 11.3
	Total	31	184.9 ± 7.2	171.7 ± 8.6	−13.2 ± 8.7
HDL cholesterol (mg/dL)	1.0	16	55.1 ± 4.6	55.2 ± 3.6*	0.1 ± 5.1
	0.3	15	52.6 ± 3.3	46.2 ± 2.4*	−6.4 ± 3.8
	Total	31	53.9 ± 2.8	50.8 ± 2.3	−3.0 ± 3.2
Triglycerides (mg/dL)	1.0	16	44.5 ± 5.4	53.5 ± 6.3	8.9 ± 4.7
	0.3	15	37.6 ± 2.7†	55.3 ± 5.0†	17.6 ± 5.0†
	Total	31	41.2 ± 3.1‡	54.3 ± 4.0‡	13.2 ± 3.4‡

* $p = 0.048$, two-tailed t test of P:S group difference.
† $p = 0.003$, two-tailed paired t test of high fat-low fat difference.
‡ $p = 0.001$, two-tailed paired t test of high fat-low fat difference.

L), 95% CI = 6.5 to 19.9, $p = 0.001$) with the increase most pronounced in the P:S = 0.3 group (17.6 mg/dL [0.20 mmol/L]).

Values for the P:S groups within study periods were similar except for a significantly lower mean HDL in the low-fat period for women on the P:S = 0.3 diet (difference = −9.0 mg/dL [−0.23 mmol/L], $p = 0.048$).

Discussion

The premenopausal women examined in this study showed only slight decreases in total plasma cholesterol when shifted from a high-fat to a low-fat diet. This was a healthy group, not chosen to be representative of US women in this age group, and this is reflected in their low baseline plasma cholesterol value (mean = 168 mg/dL [4.34 mmol/L]) compared with the estimated value for US women aged 18–44 yr (mean = 203 mg/dL [5.25 mmol/L]) (15). The lack of a significant difference in total plasma cholesterol values between the high- and low-fat periods was not a result of an inability to detect such an effect (ie, low power). The power for statistical testing was 0.90 to detect a 10% change from the baseline total sample mean cholesterol value (a change of 17 mg/dL [0.44 mmol/L]) using a two-tailed test. However, tests of the high- to low-fat differences in the HDL cholesterol fraction and tests within P:S groups did have low power (< 0.80). It is also unlikely that the lack of change in plasma cholesterol was due to poor dietary compliance. Most meals were consumed at the research site in the presence of a study dietitian. Body weight was closely monitored as an indirect measure of compliance and mean weight changed by < 1 kg between the baseline, high-fat, and low-fat periods.

While no significant response was seen in total cholesterol, the pattern of the results was consistent with current knowledge that replacing fat in the diet with carbohydrate will drop plasma cholesterol levels (16, 17) and that high P:S diets will produce lower cholesterol levels than low P:S diets at the same total fat intake (18, 19). These patterns were not evident in the HDL-cholesterol results. When switched from the high- to the low-fat diet, the HDL fraction remained constant for women in the high P:S group. In contrast, over half of the decrease in total cholesterol in the low P:S group was attributable to a decrease in mean HDL cholesterol. As a result there was a significantly lower mean HDL cholesterol in the P:S = 0.3 group compared with the P:S = 1.0 group in the low-fat period.

The changes observed in the high P:S group of our study are in general agreement with those reported by Weisweiler et al (5). In their analysis of 22 normolipidemic women fed 42% fat P:S = 1.0 and 32% fat P:S = 1.0 diets, a slight decrease in total cholesterol and no significant difference in HDL cholesterol were evident. The fall in HDL cholesterol observed on the low P:S low-fat diet in our study was also noted by Brussaard et al (7). They reported a decrease of 4% in HDL cholesterol when normolipidemic males and females were changed from a 30% fat P:S = 1.0 diet to a 20% fat P:S = 0.4 diet. An earlier study by this group (8) did not observe a decrease in HDL, but the comparison low-fat group in that study had a lower baseline mean HDL level which may have made any subsequent drop difficult to detect. Some of the women in Brussaard's study were taking oral contraceptives and no effort was made to synchronize blood drawing across the menstrual cycle, possibly confounding the results, because significant changes in plasma cholesterol (similar to the 12 mg/dL [0.31 mmol/L] difference observed in this study) occur during the menstrual cycle (20, 21).

Kuusi et al (22) examined the effect of both high P:S (0.9) and low P:S (0.4) low-fat (23% kcal) diets on plasma lipids in 44 couples in North Karelia, Finland and found decreases in HDL cholesterol in both P:S groups for women and men after 6 wk on the low-fat diet. No differences in response due to P:S group were noted. It is possible that this result differed from ours because of subject characteristics. Women in the Kuusi study were older (35–49 yr) and had higher baseline cholesterol levels (mean of 230 mg/dL [5.95 mmol/L]). In addition, both Finnish study groups increased their P:S ratios during the intervention period from a baseline P:S = 0.2. These researchers have suggested that the decrease in HDL observed during their low-fat high P:S diet may have been a transient effect, because the Oslo study (23) demonstrated a decrease in total cholesterol with an increase in HDL

cholesterol after 4 yr using a similar diet. Although the subjects in our study were only on the low-fat diet for ~1 mo longer than in the intervention reported by Kuusi et al, it is possible that transient effects may occur more quickly and last for a shorter time in younger, healthier women.

In contrast to the small changes seen in plasma cholesterol values, the mean plasma triglyceride level increased by 32% for the total group between the high- and low-fat periods. This increase was most marked (47%) in the low P:S group. The change in plasma triglycerides evident here was probably due to the shift to a high-carbohydrate diet. Sixty-four percent of calories came from carbohydrate in the low-fat diet vs only 45% on the high-fat diet. High-carbohydrate diets have been demonstrated to cause increased triglyceride levels (24, 25) through greater production of VLDL by the liver (26). Increased triglyceride levels in the VLDL fraction have been reported in other studies comparing high- and low-fat diets in normolipidemic women (4, 5). Some researchers have reported (27) that the increase in triglycerides associated with a high-carbohydrate diet peaks within a few weeks and then diminishes. However, in a study by Antonis and Bersohn (28), it took over 5 mo for the subjects to return to their baseline triglyceride values. Brussaard et al (7) reexamined the work of Antonis and Bersohn and reported that while the low P:S group finally did return to baseline levels, the high P:S group did not, which was consistent with the results of their own 16-wk study. In the work reported here, triglyceride values in women in either P:S group after ~4 mo on the low-fat diet did not return to levels observed during either the high-fat or the baseline free-living periods.

This research indicates that for healthy premenopausal women with low baseline plasma cholesterol levels a marked decrease in total-fat intake on a weight-maintaining diet is not accompanied by statistically significant changes in plasma cholesterol. Significant increases in plasma triglycerides were seen on the low-fat diet, most probably due to the replacement of calories by carbohydrate. ■

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